DETAILED ACTION

1. Claim 2 has been cancelled. Claim 7 is new.

Claim 1 and 3-7 are pending and under examination.

Response to Arguments

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1 and 3-6 remain and the new claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Beck et al. (Mechanism of Development, 1999, 88: 221-227), in view of both Witte et al. (Microscopy Research and Technique, 2001, 55: 122-145) and Bartel et al. (Anat Embryol, 2000, 202: 55-65).

Beck et al. teach a transgenic *Xenopus* comprising GFP (i.e., a reporter gene) under the control of mammalian tissue specific promoters such as the pancreatic-specific PDX-1 promoter, the liver-specific transthyretin promoter, and the small intestine-specific IFABP promoter, wherein the promoters drive specific GFP expression in pancreas, liver, and small intestine, respectively (claim 1) (Abstract, p. 221, column 2, p. 223, column 2, p 224, columns 1 and 2, p. 225, column 1). Beck et al. also teach a method of obtaining the transgenic *Xenopus* animals comprising GFP, wherein GFP

expression is driven by the promoters above (claim 3) and a method of visualizing the pancreas, liver, and small intestine by observing GFP expression in these transgenic *Xenopus* animals (claim 5) (p. 225, column 2, last paragraph, p. 226, column 1, third full paragraph).

Although Beck et al. teach that transgenic *Xenopus* animals comprising mammalian tissue-specific promoters driving GFP expression can be generally used to study later developmental stages, such as organogenesis (Abstract, p. 221, column 2, first full paragraph, p. 225, column 2, third full paragraph), they do not specifically teach making transgenic *Xenopus* comprising GFP under the control of promoters specific for expression within the lymphatic vessels (claims 1, 3, and 4) nor do they teach using this transgenic *Xenopus* to visualize the lymphatic vessel system (claim 5) or to screen for compounds capable of modulating lymphatic vessel development (claim 6). Witte et al. teach that the lymphatic vessel development is poorly understood and suggest the use of experimental models to elucidate the mechanism of lymphatic vessel development and to develop new therapies (claim 6) (Abstract, p. 124, column 1, p. 127, column 1, p. 138, column 1). It would have been obvious to one of skill in the art at the time the invention was made, to modify the transgenic *Xenopus* of Beck et al. by using promoters driving specific GFP expression in lymphatic vessels (it is noted that the prior art teaches that *Xenopus* has lymphatic vessels, see Bartel et al., p. 59, Fig. 4) and use the resulting transgenic animals to study the development of lymphatic vessel system and to screen for agents which can modulate lymphatic vessel development as taught by Witte et al., with a reasonable expectation of success. The motivation to use

transgenic *Xenopus* and not the transgenic mice of Witte et al. is provided by Beck et al., who teach that compared to mice, *Xenopus* offers many advantages such as large number of transgenic animals in one day and visualization of GFP activity in live embryos at stages that are not accessible to mammals (p. 225, column 2, second full paragraph). The motivation to use the transgenic *Xenopus* in a method of screening is provided by Witte et al., who teach the necessity to identify agents able to modulate lymphatic vessel growth (p. 138, column 1). It is noted that, by doing so, one of skill in the art would also practice a method of visualization of the lymphatic vessel system (claim 5). One of skill in the art would have been expected to have a reasonable expectation of success in doing such because the art teaches that transgenic *Xenopus* expressing GFP or transgenes in desired tissues/organs can be successfully made and used. With respect to the limitation of comparing the effect of the tested agent by comparing treated and untreated animals (claim 6), it is noted that such a step is inherent to any method of screening for modulating agents. With respect to the limitation of the lymphatic vessel system comprising lymphatic vessels, sacs and a lymphatic heart (claim 1), such is inherent to the *Xenopus* lymphatic system. It is also noted that Witte et al. teach that the tadpoles have a lymph heart (p. 122, column 1). With respect to the limitation of the promoters recited in claim 4, Witte et al. teach that VEGFR-3 and Prox-1 are specifically expressed in the lymphatic vessels (p.124). Therefore, one of skill in the art would have known to use one of these promoters to specifically express GFP in the lymphatic vessels of *Xenopus*.

With respect to the limitation of the promoter being a *Xenopus* promoter (claim

7), it would have been obvious to one of skill in the art, at the time the invention was made to use such a promoter to achieve the predictable result of expressing GFP in the lymphatic vessels of *Xenopus*.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant traversed the instant rejection on the grounds that Beck et al. teach a transgenic *Xenopus* comprising GFP under the control of mammalian tissue specific promoters, wherein the promoters drive specific GFP expression in different parts of the gut. Applicant argues that Beck et al. do not specifically teach making transgenic *Xenopus* comprising GFP under the control of promoters specific for expression within the lymphatic vessels, nor do they teach using this transgenic *Xenopus* to visualize the lymphatic vessel system or to screen for compounds capable of modulating lymphatic vessel development.

With respect to Witte et al., Applicant argues that they teach that the lymphatic vessel development is poorly understood and describe the use of experimental mammalian models to elucidate the mechanism of lymphatic vessel development. Applicant disagrees with the Examiner's interpretations of Witte et al. Specifically, Applicant argues that, on p. 138, column 1, Witte et al. only discuss refined imaging methods (MRI, NMR, videomicroscopy) to study lymphatic structures. Additionally, in the section on experimental animal models on page 127-128 of Witte et al., no screening model has been proposed: the models serve as basis for elucidating the

mechanisms of lymphatic development. See e.g. the introduction to this section, on page 127, column 1:

> "A variety of experimental preparations either newly developed or worthy of being revisited with modern techniques hold promise to elucidate the mechanisms of lymphvasculogenesis/lymphangiogenesis and their disorders, whether exuberant (compensatory) or defective (causative)."

The word 'screen' occurs once in the section on animal models, on page 128, column 1 however this is in a different context. Specifically, this section does not refer to using animal models to screen for agents capable of modulating lymphatic vessel development, but relates to screening humans for the presence of infection by looking at the presence of live adult worms:

> "The earliest clinically detectable abnormality in filariasis is hindlimb lymphatic dilatation and collateralization related to nests of live adult worms thrashing about inside hindlimb lymphatics. These lymphangiogenic events can not only be seen invasively (Case et al., 1992a) and imaged noninvasively (Witte et al., 1988), but also can be recreated in vitro. This early lymphatic abnormality has been used to screen for productive (microfilaremic) infection (Witte et al., 1993) and also exploited to follow the efficacy of therapeutic intervention in patients in endemic areas such as northeastern Brazil (Amaral et al., 1994) and in Southern India (Suresch et al., 1997)" (page 128 of Witte et al., column 1)

Applicant notes that the cited Witte et al., 1993 reference (Arch Intern Med 153:737- 744) relates strictly to screening patients for particular symptoms, not to screening compounds for their effect in an animal model. Thus, the Examiner's assertion that Witte et al. 'teach the necessity to identify agents able to modulate lymphatic vessel growth' and that Witte et al. 'teach using mammalian models to screen for agents capable of modulating lymphatic vessel growth' is incorrect and appears based on a misinterpretation of the teachings of Witte et al.

Moreover, Applicant argues, based on the teachings of Witte et al., one of skill in the art would not have been inclined to have combined the reference with Beck et al., as

suggested by the Examiner. In the introduction of Witte et al., it is believed to be clear that amphibians do not have lymph nodes, as these appear only later in evolution:

> "As species emerged from an aquatic environment, lymph nodes first made their appearance in birds and then in mammals, interspersed between lymph collectors and structurally pose a potential site of restriction to the free percolating flow of lymph. Further refined in mammals, including man, are well-defined lymphatic segments termed "lymphangions" situated between innumerable intraluminal valves and capable of rhythmic intrinsic contractions critical to propulsion of lymph." (p. 122, paragraph spanning column 1 and 2)

It is repeatedly emphasized in Witte et al. that these lymph nodes (or, in mammals, also lymphangions, the segments between the valves) are an important factor in trafficking of immune cells, and that defects in the transport system between two valves gives rise to inflammation and -localized - edema.

> "In conjunction with interspersed lymph nodes and lymphoid organs, the lymphatic vasculature also acts as a conduit for trafficking immune cell populations." (Abstract, page 122)

> "Because lymph propulsion depends predominantly on intrinsic truncal contraction, once lymphatics become obstructed truncal contractions initially quicken but then intraluminal valves gradually become incompetent and hydrostatic pressure in the draining tissue watersheds and lymphatics rises as intrinsic truncal contractions fail to expel lymph completely." (p. 135, 2nd column)

> "Functionally, obstruction occurs at the deep truncal or lymph node level." (p. 137, 1st column)

Witte et al. conclude that the dynamic structural changes of the lymph vasculature can be imaged, even in small animal models -again stressing the importance of the nodes (here also indicated as 'lymphatic collectors'):

> "Magnetic resonance imaging provides further information on soft tissue changes in chemical composition (water, fibrous tissue and fat) and sites of pooling of lymph/edema fluid and ectatic lymphatic collectors. Experimentally, addition of magnetic contrast has produced the first lymphangiomagnetograms. These techniques (dye injection, LAS, MRI) have even been adapted to small animals, with major improvements in high-resolution imaging of lymphatic channels and nodes, making this once invisible system accessible to study even in 2-g mouse pups." (p. 137, 2nd column).

Considering that the Witte et al. reference primarily relates to lymphedema and the underlying lymphatic failure; that the authors teach that lymphedema is the result of a lymphatic obstruction, and that this obstruction occurs 'at the deep truncal or lymph node level', an ordinarily skilled person, if looking for further models, would have looked for models able to recapitulate these features, i.e. models with contractile truncal walls (a feature of lymphangions) and/or lymph nodes. The very techniques proposed by Witte et al. (lymphangiography (LAG), lymphangioscintigraphy (LAS), or lymphangiomagnetography - see Witte et al., page 136-138) aim to visualize lymphangions and lymph nodes.

Accordingly, Witte et al. describe several mammalian models. As for small animal models, Witte teaches using mouse pups, in which "high-resolution imaging of lymphatic channels and nodes" is also possible. An ordinarily skilled person would thus not have been inclined or motivated from the cited art to have used a lower vertebrate model, as it is taught that amphibians and reptiles do not have lymph nodes (these only emerged in birds), nor contractile lymphatic truncal walls/lymphangions (these are a feature of mammals). Consequently, it appears from Witte et al. that amphibians are an unsuitable model system for studying lymphedema and the underlying lymphangiogenesis, as there is no indication that they possess a system for lymph transport with 'potential sites of restriction to the free percolating flow of lymph', a feature necessary for localized obstruction of lymph flow and the resulting localized edema, and as is evident from Witte et al. only present in birds and mammals.

With respect to Bartel et al, Applicant submits that they teach that Xenopus has lymphatic vessels. Further combination with Bartel et al. does not overcome the above-noted deficiencies, as Bartel et al. suggest only the presence of lymphatic vessels - they do not disclose anything about the functionality of the vessels or about the presence of other features of the lymphatic system.

Applicant argues that the present application is the first to show a functional lymphatic system in *Xenopus* tadpoles, including lymphatic sacs (which may serve as lymphatic collectors).

Moreover, although Beck et al. mention several advantages of the *Xenopus* system, this does not imply that the system can automatically or predictably be used for other applications. In this regard, Witte et al. mention that amphibians lack lymph nodes and lymphatic vessels with intrinsic contractile truncal walls; which are the sites where obstruction (and the resulting lymphedema) occurs. Thus, even when combining Witte et al. with Beck et al., one would not have had a reasonable expectation of success to obtain an animal with functional lymphatic vessels, lymphatic sacs and a lymphatic heart, as required by the claims of the present application, particularly since all references are silent on the presence of lymphatic sacs

Stating that the ordinarily skilled person would have combined Witte et al. with Beck et al. to result in a transgenic *Xenopus* model ignores part of the teaching of Witte et al., and is thus based on an improper interpretation of Witte et al. or on inappropriate use of hindsight. At the time the invention was made, an ordinarily skilled person would not have known, for example, that lymph sacs were present in *Xenopus* and would not

have combined Witte et al. with Beck et al. because lymph nodes or similar structures are absent in *Xenopus* according to Witte et al. Moreover, the ordinarily skilled person would not have used any animal model in a method of screening based on these two references, as there is no teaching or suggestion in either reference to screen for agents able to modulate vessel growth.

With respect to the new claim 7, the same considerations apply. Applicant furthermore submits that Beck et al. provide no indication to use *Xenopus* promoters. Indeed, Beck et al. state that "only a small number of *Xenopus* promoters have been characterized in live embryos" (page 221, 1st column), and that "the fact that mammalian promoters can be used is of considerable importance for designing experiments on the development of *Xenopus* itself" (page 225, 2nd column, 3rd full paragraph) as this "will avoid the need for the cloning of endogenous promoters for misexpression studies" (ibidem).

Therefore, Applicant requests the withdrawal of the rejection.

Applicant's arguments are acknowledged; however, they are not found persuasive for the following reasons:

Applicant argues that Beck et al. do not specifically teach the claimed invention. In response to this argument, it is noted Beck et al. do not have to teach each and every claim limitation. If they did, this would have been anticipation and not an obviousness-type rejection.

Applicant argues that Witte et al. do not teach the use of experimental models to screen for compounds capable of modulating lymphatic vessel development. This is incorrect. Just because they do not use the word screening in the same paragraph they teach the animal models does not mean that Witte et al. do not teach using these animal models to screen for compounds capable of modulating the development of lymphatic vessels. Specifically, Witte et al. teach that, at the time the invention was made, animal models were used to study lymphatic vasculogenesis and lymphangiogenesis in normal and pathological conditions. They also teach that the target of using these animal models is to study the effect of preventive and treatment approaches (i.e., screening for compounds able to modulate lymphatic vasculogenesis and lymphangiogenesis) (see Abstract, p. 127, column 1, last paragraph, p.139, column 2). Witte et al. teach imaging dynamic structural changes of the lymph vasculature in small animal models in conjunction with stimulation and inhibition of lymphangiogenesis (i.e., screening) (p. 137, column 2, p. 138, column 1). Clearly Witte et al. teach using these animal models to screen for compounds capable of modulating the development of lymphatic vessels and the Examiner did not misinterpret the teachings of Witte et al., as Applicant asserts.

Applicant argues that, because Witte et al. teach that amphibians do not have lymph nodes, one of skill in the art would not combine Beck et al. with Witte et al. This argument is not found persuasive. The paragraphs cited by Applicant only relate to lymphedema and the role that lymph nodes play in lymphedema; they do not relate to lymphatic vasculogenesis and lymphangiogenesis. Just because amphibians do not

have lymph node does not mean that they do not develop a lymphatic system or that they cannot be used to study the lymphatic vessel development. Applicant did not provide any evidence that lymph nodes are essential for lymphatic vasculogenesis and lymphangiogenesis in amphibians. In fact, there is no such evidence. The prior art teaches that amphibians have a lymphatic system, i.e., lymphatic vasculogenesis and lymphangiogenesis occurs in the absence of lymph nodes (see the rejection above).

Applicant argues that the teachings of Witte et al. indicate that amphibians are not suitable for studying lymphedema and the underlying lymphangiogenesis. This argument is irrelevant to the instant rejection which relates to lymphatic vessel development and not to lymphedema and the underlying lymphangiogenesis.

Because Witte et al. teach the importance of screening for compounds capable of modulating lymphatic vasculogenesis and lymphangiogenesis and because Beck et al. teach the advantage of using *Xenopus* over animal models, one of skill in the art would have been motivated to combine the two references.

Applicant argues that Bartel et al. do not teach that *Xenopus* lymphatic vessels are functional, nor do they teach anything about the presence of other features of the lymphatic system. This argument is not found persuasive. First, just because Bartel et al. do not mention functionality, does not mean that lymphatic vessels are not functional in *Xenopus*. Why would they be there if not for serving a purpose? Regardless, Witte et al. teach that the lymphatic system in amphibians is functional (see p. 122, column 1). Furthermore, the prior art teaches other functional features of the lymphatic system in *Xenopus* tadpoles, such as lymph heart and lymph sacs (see Witte et al., p. 122,

column 1; Li et al., U.S. Patent No. 5,780,230, column 12, lines 5-7). Based on the teachings in the art as a whole, one of skill in the art would have known that *Xenopus* has a functional lymphatic system. For these reasons, Applicant's arguments that the instant application was the first to show a functional lymphatic system in *Xenopus* tadpoles and that was not predictable that the *Xenopus* system could be used as claimed are not found persuasive.

Applicant argues that Beck et al. provide no indication to use *Xenopus* promoters. This argument is not found persuasive. All that Beck et al. teach is the convenience of using mammalian promoters because they are already cloned and proven to be functional in *Xenopus*. Just because Beck et al. teach that only a few *Xenopus* promoters have been used in live embryos, does not mean that *Xenopus* promoters were not known in the art or that it would not have been within the capabilities of one of skill in the art to clone such promoters and use them for *in vivo* studies, if one would chose to do so.

Conclusion

4. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Li et al. (U.S. Patent No. 5,780,230) was cited in response to Applicant's argument that the cited art does not teach that *Xenopus* has a functional lymphatic system. Specifically, the reference provides evidence that the lymphatic system in *Xenopus* is functional.

5. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action (specifically, including the newly added claim 7 in the obviousness-type rejection above). Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILEANA POPA whose telephone number is (571)272-5546. The examiner can normally be reached on 9:00 am-5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ileana Popa/
Primary Examiner, Art Unit 1633